Method for detecting the von Willebrand factor-cleaving protease activity of ADAMTS-13

The invention relates to a diagnostic method for detecting VWF-cleaving ADAMTS-13 activity in blood plasma and other media.

Thrombotic thrombocytopenic purpura (TTP) is a disease in which the classical symptoms of thrombocytopenia and microangiopathic, hemolytic anemia, neurological symptoms, disturbances in kidney function, and fever, are observed. Unusually large multimers of the von Willebrand factor (VWF) are found in plasma from TTP patients and are regarded as being the reason for the formation of VWF-rich and platelet-rich thrombi. Endothelial cells release von Willebrand factor in the form of large multimers which, in normal plasma, are cleaved by the combined action of a reductase and a metalloprotease.

In addition, it is already known that patients suffering from congenital or acquired TTP are observed to lack a specific metalloprotease which cleaves VWF between the ~~peptide bonds~~ amino acids Tyr842 and Met843. This metalloprotease has recently been identified as a new member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family and designated ADAMTS-13 (1-3).

In that which follows, the VWF-cleaving protease activity of ADAMTS-13 is simply termed ADAMTS-13 activity. ADAMTS-13 activity is normally measured by incubating a VWF sample, which has been treated with urea or guanidium hydrochloride, with dilute plasma at low ionic strength. The proteolysis is detected by means of a multimer analysis using SDS agarose gel electrophoresis or by

14 patients in connection with 22 acute TTP attacks. The initial diagnosis was based on clinical symptoms (especially neurological disturbances) and laboratory findings such as severe thrombocytopenia and the detection of microangiopathic hemolytic anemia. Plasma samples were also obtained from 11 patients who were in remission. Blood was also obtained from 10 patients during plasma substitution therapy. Plasma samples from 23 patients suffering from acute thrombocytopenia and/or hemolysis, 14 patients suffering from antiphospholipid syndrome and 80 healthy test subjects were also analyzed. Platelet-depleted plasma was prepared by centrifuging at 2500 g for 40 minutes at 4 °C. The supernatant was then stored at -20 °C until used.

2. Using the method according to the invention to determine ADAMTS-13 activity

Plasma samples were diluted 1:21 with 5 mM Tris-HCl buffer, pH 8, which contained 12.5 mM barium chloride ($BaCl_2$) and 1 mM PefaBloc SC, a serum protease inhibitor (AppliChem GmbH, Darmstadt, Germany), and then incubated at 37 °C for 5 minutes in order to activate the protease. A purified VWF (Concendre de Facteur Willebrand Humain Tres Haute Purite, LFB France) was used as substrate. The concentrate, which was free from detectable ADAMTS-13 activity, was reconstituted with water for injection to a concentration of 100 U/ml, aliquoted out and stored at -20 °C until use. Prior to the protease acting on it, the substrate was thawed, diluted, in a ratio of 1:20, with 5 M urea in 5 mM Tris-HCl, pH 8, and incubated at room temperature for 5 minutes. 100 µl of the substrate solution were then added to 210 µl of diluted plasma and the whole was left to react overnight at 37 °C. After